weight, a T cell dose comparable to bone marrow transplant, in a complete product with the exception of incidental losses that occur during the process of T cell depletion (TCD). Following collection, CD3+ T cells are enumerated by flow cytometry and a portion of the product containing a dose of 3.0×10^7 CD3+ cells/kg is set aside. The remainder of the product is depleted of T cells by a two-step method where the cells are first labeled with the anti-CD3 monoclonal antibody OKT3 attached to ferromagnetic microspheres. The product is then incubated for 30min, washed twice, and passed through a magnetized bead matrix that isolates the CD3+ T cells (CliniMACS; Miltenyi Biotec, Duarte CA). The T cell depleted product is then combined with the set aside portion of the product containing 3.0×10^7 CD3+ cells/kg for the transplant. Three separate procedures revealed 3.2 ± 0.7 log TCD for the depleted product. Recovery of other cellular components in the TCD product was as follows: B cells 75.4% ± 17.5, NK cells 33.9% ± 30.3, CD34± cells 42.0% ± 8.0. Viability of the TCD product was 97.1% ± 1.9 and sterility assessment revealed all cultures to be free of organisms following 14 days culture. Taken together, these data confirm that partial TCD can be accomplished in a closed system allowing standardization of graft T cell content.

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THE IMPORTANCE OF GRAFT CELL COMPOSITION TO OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Stem cell dose has been shown to be important for outcome in patients who undergo stem cell transplantation (SCT). Grafts may contain varying amounts of different cell populations like CD3+, CD19+, CD56+, CD4+ and CD8+ immunocompetent cells, whose role for clinical outcome is less clear.

Patients and Methods: We analyzed 591 patients who underwent SCT at Karolinska University Hospital, Huddinge between 1998 to 2007 and correlated FACS results of the above mentioned cell populations to clinical outcome. Most patients (76%) had hematological malignancies. 58% of the patients received a myeloablative conditioning regimen, 62% were given peripheral blood stem cells (PBSC) and in 58% of the transplants an unrelated donor was used.

Results: As expected, PBSC contained much higher levels of all different cell populations as compared to BM. We found that patients reciving high numbers of CD4+, and CD8+ cells had significantly more rapid engraftment. Also, a CD4+ level above 200 × 10(6)/kg was correlated to higher risk of developing acute GVHD grades II-IV (41%) as compared to patients receiving below this level (24%) ($p<0{,}037$). While the incidence of bacteria sepsis was lower in patients receiving a high number of cell dose (CD3, CD19, CD56), no correlation between cell dose and CMV reactivation was found. Relapse free- and overall survivals were not significantly affected by the different cell populations.

Conclusion: Grafts containing high numbers of CD4+ cells is a risk factor for developing severe acute GVHD but survival rates were not significantly correlated to graft composition.

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HLA MISMATCHED MSC SUPPRESS T LYMPHOCYTE ALLORESPONSES IN VITRO AND DO NOT INDUCE IMMUNOLOGICAL MEMORY IN RECIPIENTS OF MSC INFUSION

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Multipotent mesenchymal stromal cells (MSC) are increasingly used to treat refractory graft-versus-host-disease and other complications in HLA-matched and mismatched hematopoietic stem cell transplantation (HSCT) patients. We evaluated immunogenicity of human allogeneic MSC infused post-transplant to HLA-mismatched, i.e. patients undergoing HSCT. We compared recipient lymphocyte response to MSC and peripheral blood lymphocytes (PBL) from the MSC or third party donors before and after infusion, and lymphocyte responses to MSC and to PBL from the MSC donor in primary and secondary challenge using ^{3}H-tymidine. MSC recipients displayed in median responses less than 500 counts per minute (CPM) to infused third party MSC 1 week to 6 months following infusion. However, the recipients responded normally to MSC donor lymphocytes, >1000 CPM ($p<0.005$), and third party lymphocytes, >5000 CPM ($p<0.005$). MSC failed to prime responder lymphocytes to rechallenge with PBL, as the responses was <10 000 versus >30 000 CPM for the corresponding control ($p<0.05$). On MSC rechallenge of lymphocytes primed with PBL from the MSC donor, only responses <500 CPM occurred. MSC upregulated lymphocyte gene expression of CD25, IFN-γ, FoxP3, CTLA-4 and IL-10 upon MSC/PBL co-culture and MSC presence in mixed lymphocyte cultures. Unprimed and primed responder lymphocytes expanded and proliferated poorly to MSC as stimuli, evaluated by flow cytometry. The MSC failed to induce CD25+ (activated) or CD57+ (effector) CD4+ or CD8+ subsets and only inconsistently induced FoxP3+ regulatory T lymphocytes. These results confirm *in vivo* and *in vitro* that infused MSC are weakly immunogenic and do not induce significant immunological memory in HLA-disparate recipients.

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DOUBLE CORD BLOOD TRANSPLANTATION (CBT) WITH EX-VIVO EXPANSION (EXP) OF ONE UNIT UTILIZING A MESENCHYMAL STROMAL CELL (MSC) PLATFORM

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Delayed or failed engraftment is a frequent complication of CBT. We developed an approach that involves ex vivo co-culture of CB mononuclear cells with third party marrow derived MSCs. Patients must have 2 CB units matched in at least 4/6 HLA antigens, with a minimum of 1×10^7 TNC/Kg per unit. A family member matched in at least 2/6 antigens or more serves as the marrow-derived MSC donor.

Methods: Diagnoses: AML/MDS (N = 4), ALL (N = 1), and CLL (N = 1). One patient was in CR and 5 had active disease (1 failed a previous CBT). Preparative regimen: myeloablative melphalan (140mg/m2), thiotepa (10mg/kg), fludarabine (160mg/m2), and rabbit ATG (3 mg/kg). GVHD prophylaxis: tacrolimus and MMF. Median weight was 57 Kg (range, 14–79). Donor-recipient HLA matching was 5 of 6 in 35% of the cases and 4 of 6 in 65%. Ex-vivo EXP: 100 ml of marrow was aspirated from the family donor and MSCs were generated in 10 T175 flasks. The CB unit with the lowest TNC dose was thawed, and placed into flasks containing confluent layers of MSCs in EXP media with SCF, FLT3, G-CSF and TPO. After 7 days at 37°C, the non-adherent cells were removed, and placed into one-liter Teflon-coated culture bags and cultured for an additional 7 days (14 days total); 50 ml of media/growth factors was added to the flasks to culture the remaining adherent layer during that time period. On day 14 the cells were washed and infused along with a 2nd unmanipulated unit.

Results: There were no toxicities attributable to EXP. The median fold EXP was 12 (1–13) for TNC and 12 (0–27) for CD34+ cells. Median time to neutrophil and platelet engraftment was 14.5 days (12–23) and 31 days (25–51). All patients (n = 6) engrafted neutrophils and platelets and became complete donor(s) chimeras. Chimerism: 1 unit dominated in all patients; 2 patients have mixed donors chimerism 4 months after CBT, with 5% and 20% of the EXP unit contributing to hemopoiesis. Three patients had no evidence of EXP unit-derived hemopoiesis after day +30, while 1 patient is 100% donor but the contributing unit